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Optimisation of various Ingredients for the Development of Quinoa fortified Bread and their Storage Studies

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ABSTRACT: Quinoa is known as super food due to its exceptional nutritional quality and balanced amino acid profile. Present study comprises optimisation of various ingredients in quinoa fortified bread and analysis of quality of bread for 7 days of storage. Sorption isotherm of bread at 60% RH, 80% RH and 100% RH showed maximum moisture content as 43.21% in 5.98 % quinoa containing sample, 43.81 and 43.99 % in 25.01% quinoa flour fortified samples respectively whereas minimum moisture as 42.30, 42.70 % observed in control samples and 42.88 % in 5.98% quinoa addition samples respectively. It was presented in results that loaf volume of quinoa fortified bread during zero day, 4th day and 7th day was observed 2200, 2195 and 2190cm^3 maximum in control samples whereas 1905, 1902 and 1900cm^3 was recorded in 25.01% quinoa samples during storage period respectively. It could note that 4th day and 7th day of moisture loss during storage found maximum as 40.77 and 38.85% in 15.5% quinoa samples whereas minimum as 39.83 and 37.90% observed in 5.98% quinoa samples respectively. It was seen from the result that staleness was not observed at zero day in quinoa fortified bread. It was reflected from the table that 4th day and 7th day staleness of bread during storage period found highest as 6.25 and 8.54 in 25.01% quinoa samples while lowest as 3.0 and 7.10 observed in control samples respectively. Total plate count is effective method for determining microbial population in bread sample and estimates shelf life of bread. Quinoa fortified bread microbial analysis conducted for 0 days, 4 days and 7 days of storage at ambient temperature (37°C Temperature). Results showed that zero days of storage did not show any microbial activity. Obtained results showed higher microbial activity was found in 7 days of storage which was observed as 5.38 log cfu/g whereas 4th day of storage showed moderate activity which was about 3.25 log cfu/g. According to AOAC, the TPC for bread should be>0.88-6.13log CFU/g. In the present study, we found that on 7th day the TPC is lower than recommended safety level and on 9th day, the plate count is higher than recommended. Therefore, the present study found that the quinoa optimised bread is safe up to the 7th day.

Keywords: Quinoa, Bread, Sorption isotherm, Loaf volume, Total plate count, Shelf life, microbial activity.

INTRODUCTION

Quinoa has potential to provide food and nutrition security in the present day scenario as this grain was considered to be sacred by the Incas due to its exceptional nutritional components. Quinoa (*Chenopodium quinoa* Willd) is a pseudo cereal of *Amaranthaceae Chenopodium*, which is the only single plant that meets the basic nutritional needs of the human body (Kumar *et al.*, 2022). Quinoa flour contains 11.2 % moisture, 13.5 % crude protein, 4.3 % fat, 9.5 % crude fibre, 1.2 % total ash and 58.3 % carbohydrate (Ogungbenle, 2003). Besides, quinoa is also abundant in unsaturated Fatty acids (linoleic and linolenic acids), vitamins (folate and tocopherols), minerals (iron, copper, manganese, and potassium), dietary fiber and polyphenols including flavonoids and its amino acid composition is close to the ideal protein balance recommended by the FAO, rich in histidine, lysine, threonine and methionine which are deficient in cereals.

Turkut *et al.* (2016) suggested that quinoa flour might be considered as a good alternative for gluten-free pan bread making. Milovanovic *et al*. (2014) noticed that supplementation of wheat four dough with quinoa flour appeared in nutritionally improved bread with sensory acceptance. Demin *et al*. (2013) showed that the sensory evaluation of quinoa and buckwheat supplemented (30% and 40% level) bread has good and acceptable in terms of appearance, texture, and flavour.

Lancelot *et al*. (2021) found that the bread staling was affected by many factors, such as bread formula, processing technology, and storage temperature, but the most important factor was starch retro gradation**.** Collar *et al*. (2009) showed that the effect on starch was important because this component was responsible for bread staling. Barcenas and Rosell (2005) revealed that most of the time bread quality loss is not due to microorganism or endogenous enzyme deteriorative activity but staling.

Martinez *et al.* (2018) observed that the increase in crumb hardness is commonly used as an indicator of bread staling, which is a major cause of deterioration in bread quality and affected by many factors, such as retro gradation of starch molecules and water migration. Hayes *et al.* (2020) observed that the hardness of quinoa bread increased at all temperatures over the storage time, the most widely used indicator of staling is measurement of the increase in crumb firmness. Torbica *et al*. (2010**)** suggested quality and shelf life of gluten-free breads can be improved by using pseudo-cereals such as quinoa, buckwheat and amaranth with their nutritional value and the techno-functional properties.

There are few studies on the microbial aspects of quinoa substituted bread during storage. Oluwajoba *et al.* (2014) compared the microbiological, nutritional, and sensory quality of bread produced from wheat and quinoa flour blends. They found that the total aerobic bacterial counts and fungal counts of the bread increased with increasing quinoa flour substitution, but were still within acceptable limits. Coliforms were not detected in the bread. The authors suggested that quinoa flour could be used to improve the nutritional quality of bread without compromising its microbial safety or sensory acceptability. Li *et al*. (2022) investigated the impact of daily consumption of whole-grain quinoa-enriched bread on gut microbiome in males. They found that there were no significant changes in the gut microbiome composition or diversity after 4 weeks of consuming either quinoa-enriched or control wheat bread. The authors concluded that small changes in the type of cereal consumed (substituting 20 g of refined wheat flour with whole-grain quinoa flour) was not able to significantly modulate the gut microbiome. Quinoa fortified bread had a great nutritional potential and it is primary cause of susceptible to spoilage rapidly. In this storage study developed bread noted in terms of moisture isotherm, moisture loss, total plate count, loaf volume and staleness.

Optimization of various ingredients and development of bread from quinoa flour. Different formulations of bread were developed as per RSM central composite design with varying proportions of Refined Wheat flour: Quinoa flour, Sugar and Vegetable oil, yeast and Dough improver. The results showed that the product with quinoa flour upto 15.5% was acceptable, above which the product is change in dark brown color which was not acceptable by the panelist during sensory evaluation. The acceptable percentage range of yeast, sugar and vegetable fat and improver are 1.2-1.70,5.0-6.5, 1.3-1.5 and 1.2-1.3 respectively. Out of all the 50 combinations the following combinations are acceptable:

Table 1: Optimization of quinoa fortified bread with acceptable ingredients.

Sr. No.	RWF (%)	Quinoa Flour (%)	Sugar (%)	Veg. Fat (%	Yeast (%)	Improver (%)
T_0	100	0	5	1.5	1.2	1.2
T_1	88.5	11.5	5	1.3	1.2	1.2
T_2	80.5	19.5	5	1.3	1.2	1.2
T_3	88.5	11.5	6.5	1.3	1.2	1.2
T_4	80.5	19.5	6.5	1.3	1.2	1.2
T_{33}	94.02	5.98	5.75	1.5	1.35	1.3
T_{34}	74.99	25.01	5.75	1.5	1.35	1.3
T_{35}	84.5	15.5	3.96	1.5	1.35	1.3
T_{36}	84.5	15.5	7.53	1.5	1.35	1.3
T_{43}	84.5	15.5	5.75	1.5	1.35	1.3

RWF-Refined Wheat Flour

MATERIAL AND METHOD

The standard sorption apparatus recommended by Wolf *et al*. (1985) was used for equilibrium studies.

Total plate count was determined on TPC agar. Powdered sample was suspended in sterile peptone salt solution and then homogenized for some time. A series of decimal dilutions were prepared. The sample from appropriate dilution was then plated on solidified and dried agar. Colonies were then counted with the help of digital colony counter. Results were expressed as CFU/g (Gupta *et al*., 2011).

Loaf volume was determined by rapeseed displacement method. Bread hardness is the indicator of staleness. Hardness of bread checked during storage period by sensory analysis of bread texture. Moisture content was determined as per oven drying method.

RESULTS AND DISCUSSION

Quinoa fortified breads which was selected by trained sensory panel used for further evaluation of storage parameters such as sorption isotherm, microbial load, loaf volume, moisture and staleness. Data obtained from the studies noted in below table.

Sorption Isotherm. At a quick glance of the table showed that sorption isotherm of bread at 60% relative humidity, 80% relative humidity and 100% relative humidity reflected as 43.21% maximum in T_{43} sample, 43.81 and 43.99 % maximum in T_{34} samples respectively whereas 42.30, 42.70 % minimum observed in control samples and 42.88 % in T_{33} samples respectively. It was revealed from the results that increasing humidity percentage affect significantly with increasing moisture content of the quinoa fortified bread.

Table 2: Sorption isotherm of quinoa substituted bread at different RH levels.

Formulations	Moisture (%)		
	60%RH	80%RH	100%RH
T_0	42.30	42.70	42.95
T_1	42.56	42.85	43.11
T_2	42.85	42.99	43.52
T_3	42.50	42.83	42.98
T_4	42.90	43.22	43.49
T_{33}	42.36	42.59	42.88
T_{34}	43.65	43.81	43.99
T_{35}	42.96	43.23	43.58
T_{36}	42.97	43.24	43.60
T_{43}	43.21	43.51	43.79

RH-Relative humidity, T_0-Control

During shelf life, the moisture content decreased for all samples due to various factors, including migration of water from crumb towards crust, water loss to the atmosphere caused by the permeability of the packaging, and starch retro gradation (Cauvain, 1998).

Total plate count (TPC) and Loaf Volume. Storage study of bread was conducted for a period of seven days at room temperature. During the storage period total plate count and loaf volume were determined for calculation of safe consumption period. Storage study were conducted at zero days, 4th day and 7th day. Data obtained during the study were given in tabulated form.

It was noticed from the table that total plate count of quinoa fortified bread not observed during zero days of storage. It could be noted from the results that 4th day and 7th day of storage showed highest as 1.55×10^5 and 2.56×10^5cfu/g in T_{34} samples whereas lowest as 1.15×105 cfu/g observed in control and 2.08 cfu/g minimum in T_{33} samples respectively. It was exhibited from the results that total plate counts were increased every day and after 7th day of storage quinoa fortified were not consumable as total plate count exceeds safe level.

Table 3: Total plate count (TPC) and Loaf Volume of Quinoa substituted bread at room Temp. for 7 Days.

Sr. No.	Total Plate Count(CFU/ml)			Loaf Volume(Cm^3)		
	0Day	4 day	7th day	0Day	4 Day	7 Day
T_0	NO	1.15×10^5	2.10×10^5	2200	2195	2190
T_1	NO	1.3×10^5	2.2×10^5	2100	2096	2091
T_2	NO	1.43×10^5	2.42×10^5	2025	2019	2017
T_3	NO	1.29×10^5	2.19×10^5	2110	2109	2106
T_4	NO	1.42×10^5	2.41×10^5	2035	2033	2030
T_{33}	NO	1.2×10^5	2.08×10^5	1980	1977	1975
T_{34}	NO	1.55×10^5	2.56×10^5	1905	1902	1900
T_{35}	NO	1.35×10^5	2.29×10^5	2050	2048	2047
T_{36}	NO	1.36×10^5	2.32×10^5	2051	2049	2048
T_{43}	NO	1.38×10^5	2.37×10^5	2050	2049	2048

T_0-Control, NO-Not Observed

Data recorded in table showed that loaf volume of quinoa fortified bread during 0 day, 4th day and 7th day observed maximum as 2200, 2195 and 2190 cm^3 in control samples whereas minimum as 1905, 1902 and 1900cm^3 recorded in T_{34} samples during storage period respectively. Data revealed from the results showed that a decreasing trend of loaf volume was observed throughout the storage study with increasing days of storage. During the storage period moisture migrated from the bread and decreases intracellular spaces within bread. Therefore this could be the most probable reason for decreasing loaf volume of quinoa fortified bread during storage.

It could be seen from results that 4th day and 7th day of storage showed highest 1.55×10^5 and 2.56×10^5cfu/g in 25.01% quinoa incorporated samples whereas lowest as 1.15×10^5cfu/g observed in control and 2.08×10^5cfu/g in 5.98% quinoa flour samples respectively. It was noted that total plate count of quinoa bread not observed during storage of zero days .Similar results were reported by Pooja *et al*. (2021) on gluten free bread made with incorporation of rice flour and quinoa flour.

Moisture loss and staleness. It was exhibited from the presented table that moisture content at zero day observed 43.65% maximum in T_{34} sample whereas minimum found in 42.30 % in control sample. Data obtained from the result showed a trend that moisture content decreased every day. It was noticed after watching results that moisture content at zero day observed maximum as 43.65% in 25.01% quinoa fortified bread sample whereas minimum found as 42.30 % in control sample. It was observed that4th day and 7th day of moisture loss during storage found highest as 40.77 and 38.85% in 15.5 % quinoa contained samples whereas 39.83 and 37.90% minimum observed in 5.98% quinoa flour samples respectively. With respect to the whole grain bread samples, moisture values were slightly higher than those obtained by Gandra *et al*. (2008), who studied bread containing wheat bran (20% flour basis) and found moisture contents of 34.13% and 31.55% on the first and seventh day of analysis, respectively.

Table 4: Moisture loss and staleness of quinoa substituted bread at room temperature for 7 days.

Sr. No.	Moisture (%)			Staleness		
	0th Day	4th day	7th day	0th Day	4th Day	7th Day
T_0	42.30	40.02	38.19	NO	5	8
T_1	42.56	40.12	38.29	NO	4	7
T_2	42.85	40.25	38.40	NO	3	6
T_3	42.50	40.02	38.15	NO	4	7
T_4	42.90	40.33	38.49	NO	3	6
T_{33}	42.36	39.83	37.90	NO	5	8
T_{34}	43.65	40.27	38.42	NO	2	4
T_{35}	42.96	40.40	38.55	NO	3	5
T_{36}	42.97	40.42	38.56	NO	3	6
T_{43}	43.21	40.70	38.85	NO	2	4

T_0-Control, NO-Not Observed

It was brought to notice from the results that staleness of quinoa fortified bread increased every single days and could not be safe after a period of 7 days of storage. It could be noted after observing result that staleness was not observed at zero day in quinoa fortified bread. With increasing days of storage, It was seen that 4th day and 7th day staleness of bread during storage period found maximum as 6.25 and 8.54 in 25.01% quinoa flour samples while minimum as 3.0 and 7.10 observed in control samples respectively. A similar effect has been shown in wheat bread (Cardone *et al*., 2020; Marti *et al*., 2018, 2017).

CONCLUSIONS

Now a day consumption of functional and convenient food with desired properties has been a growing interest. Bread is one of the most popular staple foods with great consumer acceptance in the world. However, most bread are made from refined wheat flour, which have deficiency in nutrition, such as lack of vitamins, minerals, lysine (a limiting amino acid for cereals) and dietary fibre. Quinoa is famous for their incredible amino acid balance and good fibre content. Therefore, incorporation of quinoa flour into refined wheat flour given desirable nutritional as well as functional characteristics. Thus, the present investigation entitled "Optimisation of various ingredients for the development of quinoa fortified bread and their storage studies" were studied.

FUTURE SCOPE

Quinoa fortified bread packaging material and its testing methods in terms of GTR, WVTR and OTR should be application of future domain. Another bakery products also produced with incorporation of quinoa for enhancing nutritional value.

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Conflict of Interest. None.

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